

A retrospective comparison of allogeneic peripheral blood stem cell and bone marrow transplantation results from a single center: a focus on the incidence of graft-vs.-host disease and relapse

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(Received 4 June 1998; accepted 4 December 1998)

ABSTRACT

To detect the effect of the stem cell source, allogeneic peripheral blood stem cell transplantations (alloPBSTs) performed between 1995 and 1997 from human leukocyte antigen (HLA)-identical siblings in 40 patients with acute and chronic hematological disorders were compared with a historical group of 40 patients with similar variables who had received allogeneic bone marrow transplants (alloBMTs) between 1993 and 1995. Patients in both groups were identical except that both the recipient and the donor ages were, on average, higher in the alloPBST group (26 vs. 36 [$p = 0.005$] and 27 vs. 32 [$p = 0.024$], respectively). Patients received similar therapy excluding posttransplant granulocyte colony-stimulating factor administration (97% in alloBMT vs. 12.5% in alloPBST). The median time to reach neutrophil counts $>0.5 \times 10^9/L$ and platelet counts $>20 \times 10^9/L$ was 13 and 14 days, respectively, in patients receiving alloPBSTs compared with 19 and 27 days in patients receiving alloBMTs ($p = 0.0014$ and $p = 0.0002$). The alloPBST group required similar transfusions of red blood cells or platelets. The incidence of grade II–IV acute graft-vs.-host disease (aGVHD) was similar in both groups. However, chronic GVHD (cGVHD) of all grades developed in 78.1% of patients in the alloPBST group after a median follow-up period of 12.5 (range 0.5–34) months. In alloBMT recipients, cGVHD of all grades developed in 21.4% after a median follow-up period of 38 (range 0.5–62) months ($p = 0.00001$). Day 100 transplant-related mortality was also similar: 20% (8 of 40) in the alloBMT patients and 17.5% (7 of 40) in the alloPBST group. Although not statistically significant, a relatively higher relapse rate occurred in the alloBMT group (21.4 vs. 10.7%). The estimated disease-free survival in month 24 was 51.3% for alloBMT and 54.6% for alloPBST, and the estimated overall survival in month 24 was 56.1% for alloBMT and 64.6% for alloPBST. In conclusion, this retrospective comparison suggests that alloPBST from HLA-identical donors is associated with faster engraftment, fewer transfusions, and no greater incidence of aGVHD, but a high incidence of cGVHD.

KEY WORDS

Transplantation • Bone marrow • Peripheral stem cell

INTRODUCTION

Over the last few years there has been an increase in the use of allogeneic peripheral blood stem cell transplantation

(alloPBST) to provide hematological rescue after myeloablative therapy for the treatment of various advanced hematological malignancies [1]. Although the first results are encouraging, the cell content of the infused product and its effect on engraftment and immunological recovery are still under discussion [1–4]. Advantages of using PBSTs

include the relative ease of collection and the rapid hematological reconstitution compared with those with bone marrow (BM). High doses of CD34⁺ cells obtained by PBSC mobilization improve the speed of hematopoietic recovery [1–5]. On the other hand, a high number of T lymphocytes and natural killer (NK) cells in the peripheral blood compared with BM may produce more acute graft-vs.-host disease (aGVHD) and chronic GVHD (cGVHD) [6]. Some have reported a high incidence of cGVHD without comparing similar marrow-transplanted patients [7–10]. Similarly, another group, also without comparing a matched BMT group, reported a 27% aGVHD (grade II–IV) and 47% cGVHD after alloPBSCT [11].

In this study, we retrospectively compared 40 patients receiving human leukocyte antigen (HLA)-identical sibling PBSCs with a historical group of 40 patients receiving BM for the treatment of hematological disorders. This study included chronic myelogenous leukemia (CML) patients in first chronic phase (within 12 months of diagnosis) and acute nonlymphoblastic leukemia (ANLL) patients in first or second complete remission (CR). The end points included time to engraftment, transfusion requirements, the incidence of aGVHD and cGVHD, infectious episodes, and the hematological, cytogenetic, and molecular relapse rate.

PATIENTS AND METHODS

Patient population

Forty patients who underwent alloPBSCT in our department between 1995 and 1997 were compared with a similar historical group of 40 matched allogeneic bone marrow transplantation (alloBMT) patients studied between 1993 and 1995. There were no statistically significant differences between the patients in the groups who underwent alloBMT and alloPBSCT regarding sex mismatch or ABO blood group mismatch, diagnosis, and the amount of time from diagnosis to transplantation (Table 1). Recipient ages and donor ages were higher in the alloPBSCT group than in the alloBMT group (26 and 27 vs. 36 and 32; $p = 0.005$ and 0.024 , respectively). The characteristics of the patients are summarized in Table 1. All patients were given transplants from their HLA-identical siblings and received the same conditioning regimen: busulfan, cyclophosphamide, and GVHD prophylaxis. All patients and donors were similar in terms of cytomegalovirus (CMV) serology (immunoglobulin [Ig]G-positive and IgM-negative).

Peripheral stem cell collection from healthy donors

Granulocyte colony-stimulating factor (G-CSF), used to prime the donors, was given at different doses because of several ongoing studies on the effect of escalated doses of G-CSF in healthy donors. Therefore, two patients received $2.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ subcutaneously for 10 days, three received $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 5 days, 30 received $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 5 days, and five received $15 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 5 days.

PBSC collections

In the 10-day schedule of G-CSF, leukapheresis was started on Day 5 or 6. PBSCs were collected by apheresis from normal donors using continuous flow blood separation (Cobe Spectra, COBE BCT, Lakewood, CO; or Fenwall CS

3000, Baxter Healthcare Systems, Deerfield, IL). Donor venous access was obtained through venipuncture of both arms. No central venous line insertion was needed. Further processing was not performed on the collected material. Median leukapheresis count was 2 (range 1–5). Yields were transfused the same day they were collected. The first infusion day was accepted as Day 0.

Analyses

PBSC enumeration was done using a procount progenitor cell enumeration system kit (BDIS, San Jose, CA). X-Y chromosome detection was done in 24-hour cultured lymphocytes using denaturation with a DNA probe (Vysis GmbH, Stuttgart, Germany) as described by the manufacturer. To detect chimerism after transplantation, recipients' blood, epithelial cells found in saliva, and donors' blood cells were used as samples. The method of variable nontandem repeats (VNTR) previously has been described in detail [12].

Prophylaxis and treatment of GVHD

Diagnosis and grading of aGVHD was based on accepted clinical criteria. Patients were evaluated for aGVHD if they survived 21 days and had evidence of engraftment. Cyclosporin A (CsA) and short-term methotrexate (MTX) were used for GVHD prophylaxis in all patients. CsA was administered between Day –1 and 180, and was continued if GVHD was detected. MTX was initiated on Day 1 as a dose of $15 \text{ mg}/\text{m}^2$ and was given on Days 3, 6, and 11 as a dose of $10 \text{ mg}/\text{m}^2$. When clinical grades III–IV GVHD were established, a high-dose methylprednisolone ($10\text{--}20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ i.v. with gradual tapering) was added. ATG or ALG was added to this treatment if no improvement was observed. Chronic GVHD was treated with CsA plus methylprednisolone.

Engraftment, relapse, and graft failure

Granulocyte engraftment was defined as the first of 3 consecutive days with a granulocyte count of $>0.5 \times 10^9/\text{L}$. Platelet engraftment was defined as an unsupported platelet count of $>20 \times 10^9/\text{L}$ on 3 consecutive days. Sex or blood group mismatches and VNTR polymorphism were used for detection of engraftment origin and monitoring. Patients who had hematological evidence of primary disease were accepted as having hematological relapse. In patients with CML, Philadelphia positivity starting from 12 months, conventional cytogenetics, and fluorescence in situ hybridization (FISH) or VNTR were accepted evidence of cytogenetic or molecular relapse. In cases of acute leukemia, sex and blood group mismatches and VNTR polymorphism were helpful in determining relapse. Graft failure was defined as the failure of neutrophil and platelets in engraftment in the first 28 days after transplantation.

Statistics

Differences between these two groups' recipient and donor ages, time to transplantation, mononuclear and CD34⁺ cell counts, febrile neutropenic days, duration of antibiotic therapy days, red blood cell (RBC) transfusion, and platelet transfusion were compared using the Wilcoxon rank-sum (Mann-Whitney) test. The Chi-square test was used to compare differences in the two groups for recipient and

Table 1. Patient characteristics

	Group I (BMT)	Group II (PBSCT)	p
Number of patients	40	40	
Recipient age (years [median])	26 (16–48)	36 (16–44)	0.005
Donor age (years [median])	27 (13–47)	32 (16–46)	0.024
Recipient sex (male/female)	25/15	21/19	>0.05
Donor sex (male/female)	21/19	20/20	>0.05
Sex mismatch	12	18	>0.05
ABO blood group mismatch	18	18	>0.05
Diagnosis			
AML			
CR 1	17	16	—
CR 2	0	3	—
Total	17	19	>0.05
CML (chronic phase)	17	18	>0.05
ALL			
CR 1	2	2	—
CR 2	1	0	—
Total	3	2	—
MDS	2	1	—
Adrenoleukodystrophy	1	0	—
Time to TX time (median months)	5.5 (5–16)	7.5 (4–15)	>0.05
TX year			
1993	6	0	—
1994	23	0	—
1995	11	5	—
1996	0	13	—
1997	0	22	—

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; BMT, bone marrow transplantation; CML, chronic myelogenous leukemia; CR, complete remission; MDS, myelodysplastic syndrome; PBSCT, peripheral blood stem cell transplantation; TX, transplantation.

donor sex mismatch, ABO mismatch, diagnosis, transplantation year, aGVHD grade I–II and grade III–IV, cGVHD limited and extensive, relapse, transplant-related mortality, and causes of death. The probabilities of neutrophil and platelet recovery, disease-free survival (DFS), and overall survival (OS) were compared using the method of Kaplan-Meier with log-rank analysis. Cox regression analysis was performed to detect the hazard ratio for developing cGVHD.

RESULTS

PBSC collections and engraftment

The median number of collected mononuclear cells (MNCs) and CD34⁺ cells in the PBSC and the BM group were 5.5 vs. 1.9×10^8 /kg and 5.4 vs. 3.2×10^6 /kg ($p = 0.001$ and 0.052), respectively (Table 2). Six patients in the alloPBSCT group received $<3 \times 10^6$ /kg CD34⁺ cells, two died due to sepsis with aplasia. Although 97% of the patients in the alloBMT group and only 12.5% of the patients in the alloPBSCT group received G-CSF following transplantation until engraftment, neutrophil and platelet recovery were faster in the alloPBSCT group than in the alloBMT group (13 vs. 19, $p = 0.0014$; 14 vs. 27, $p = 0.0002$; respectively) (Table 2, Figs. 1 and 2). A median of 2 units of RBCs and 4 units of platelets, and 1 unit of RBCs and 3 units of platelets were needed for the alloBMT and the alloPBSCT group, respectively ($p = 0.15$ and $p = 0.10$; Table 2). Graft failure occurred in one patient in each group.

Chimerism studies

Complete chimerism (CC) was documented in the majority of our patients (Table 3). Sex chromosome-based FISH and conventional cytogenetics demonstrated CC in 60.0% (20 of 33) of sex-mismatched patients. ABO blood typing revealed conversion of blood groups to donor type in 86.1% (31 of 36) of relevant patients. Molecular methods used to demonstrate chimerism could be applied. VNTR was uninformative in the four alloBMT sample pairs studied. However, CC was detected in 80% (12 of 15) of the patients in the alloPBSCT group.

aGVHD and cGVHD

The occurrence of aGVHD, either grades \leq II or \geq II, was similar in both alloBMT and alloPBSCT groups (Table 2). Five patients died of aGVHD in the alloBMT group, but none in the alloPBSCT group (Table 4). The incidence of cGVHD was 78.1% in the alloPBSCT group compared with 21.4% in the alloBMT group (cumulative hazard rate 2.26 ± 0.10 , $p = 0.0007$; Table 2 and Fig. 3). Twenty-one patients with cGVHD in the alloPBSCT group had hepatic involvement (Table 5), eight of whom were confirmed as having cGVHD by liver biopsy. One patient with cGVHD of the liver died of hepatic failure and three died of pulmonary infection secondary to immunosuppression for GVHD. Two patients (28.5%) in the alloBMT group and seven patients (51.8%) in the alloPBSCT group developed aGVHD before cGVHD. *De novo* cGVHD occurred after a median of 5

Table 2. Results

	Group I (BMT)	Group II (PBSCT)	<i>p</i>
MNCs $\times 10^8$ (median)	1.9 (0.35–28.7)	5.5 (2.5–38.20)	0.001
CD34 $\times 10^6$ (median)	3.2 (2.15–7.80)	5.4 (0.8–12.4)	0.052
CD3 ⁺ $\times 10^8$ (median)	—	1.85 (0.45–4.8)	—
CD4 ⁺ $\times 10^8$ (median)	—	1.11 (0.2–2.82)	—
CD8 ⁺ $\times 10^8$ (median)	—	0.96 (0.21–2.27)	—
NK cells $\times 10^8$ (median)	—	0.3 (0.16–1.01)	—
Neutrophils $>0.5 \times 10^6/L$ (days [median])	19 (12–50)	13 (10–32)	0.0014 ^a
PLTs $>50 \times 10^6/L$ (days [median])	27 (13–70)	14 (11–54)	0.0002 ^a
Febrile neutropenic (days [median])	4 (0–17)	3 (0–18)	0.27
Duration of antibiotic therapy days (median)	12 (0–38)	10 (0–30)	0.62
RBC transfusion (median)	2 (0–28)	1 (0–12)	0.15
PLT transfusion (median)	4 (1–25)	3 (1–26)	0.10
aGVHD			
Grade I–II	15.4% (6/36)	17.1% (6/35)	0.957
Grade III–IV	13.9% (5/36)	14.3% (5/35)	0.961
Total	30.6% (11/36)	31.4% (11/35)	0.936
cGVHD			
Limited	17.9% (5/28)	59.3% (19/32)	0.001
Extensive	3.6% (1/28)	18.8% (6/32)	0.067
Total	21.4% (6/28)	78.1% (25/32)	0.00001
Relapse	21.4% (6/28)	10.7% (3/28)	0.275
Disease-free survival (median)	34 (0–62)	12 (0–34)	0.92 ^a
Follow-up month (median)	38 (0.5–62)	12.5 (0.5–34)	0.81 ^a

^aLog-rank.

aGVHD, acute graft-vs.-host disease; cGVHD, chronic GVHD; MNCs, mononuclear cells; NK cells, natural killer cells; PLTs, platelets; RBC, red blood cells.

months (range 3–11 months) in the alloBMT patients and 6.5 months (range 3.5–11 months) in the alloPBSCT patients. Chi-square analysis revealed a higher incidence of cGVHD in patients with prior histories of aGVHD ($p = 0.02$).

The CD4/CD8 ratio decreased in alloPBSCT patients during the follow-up period. The median ratio was 1.6 (range 0.22–1.95) before transplantation and 0.3 (range 0.21–0.52) in the 12th month posttransplant. The median percentages of CD3⁺ lymphocytes in whole lymphocyte cells were 23 and 53% in the 1st and 12th months, respectively.

Infections

Febrile neutropenic days numbered 4 (range 0–17 days) in the alloBMT group and 3 (range 0–18 days, $p = 0.62$) in the alloPBSCT group (Table 2). The duration of antibiotic therapy was 12 days (range 0–38 days) in alloBMT patients and 10 days (range 0–30 days) in alloPBSCT patients ($p = 0.27$). Veno-occlusive disease occurred in one patient in each group.

Graft rejection

One patient in the alloPBSCT group underwent transplantation 2 years after the diagnosis of CML. VNTR analysis revealed recipient cells at the 2nd month and ABO blood typing showed the patient's ABO blood group type at the 4th month.

Outcome and relapse

The overall relapse rate was 21.4% in the alloBMT group and 10.7% in the alloPBSCT group ($p > 0.05$;

Table 2). Following alloPBSCT, two patients with acute myeloblastic leukemia (AML) and one patient with CML relapsed (Table 2). One of them has received a transplant at her second CR 6 years after the initial diagnosis. At the 2-month evaluation, despite hematological CR, VNTR revealed rejection. At the 4-month evaluation, however, her ABO blood group changed to donor type. At the 8-month evaluation, marrow blasts were in 40% of BM cells and VNTR analysis revealed rejection again. She died during reinduction chemotherapy. Another patient relapsed and plasma cell leukemia was confirmed by morphological immunophenotyping and VNTR analysis, which showed conversion of CC to mixed chimerism (MC) on peripheral blood and BM cells. The patient is still alive and in her second CR 5 months after donor lymphocyte infusion. Neither patient developed either aGVHD or cGVHD. The third patient who underwent alloPBSCT for the treatment of CML relapsed as blastic crisis 12 months after the procedure while he was suffering from extensive cGVHD. He did not receive any chemotherapy because of the cirrhosis that could result from either Hepatitis B virus infection or cGVHD.

In the early phase posttransplantation, VNTR showed rejection in two patients with CML. One patient developed hematological and cytogenetical remission and had the donor's blood group 8 months after alloPBSCT. She died because of cGVHD. VNTR failed to show CC twice for another patient who had a long time between diagnosis and transplantation. She was diagnosed as having graft rejection and died in the 8th month.

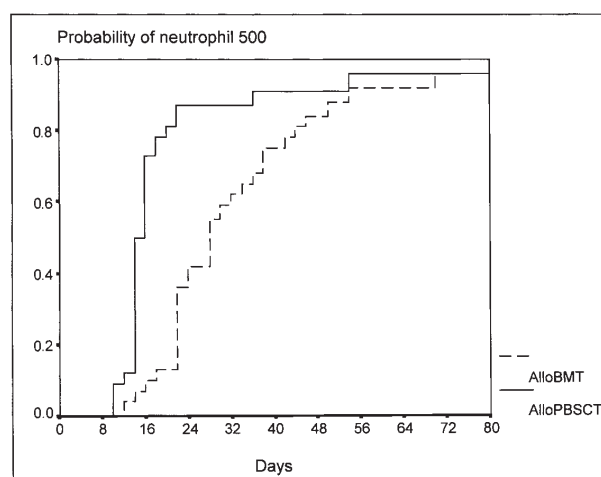


Figure 1. Neutrophil engraftment

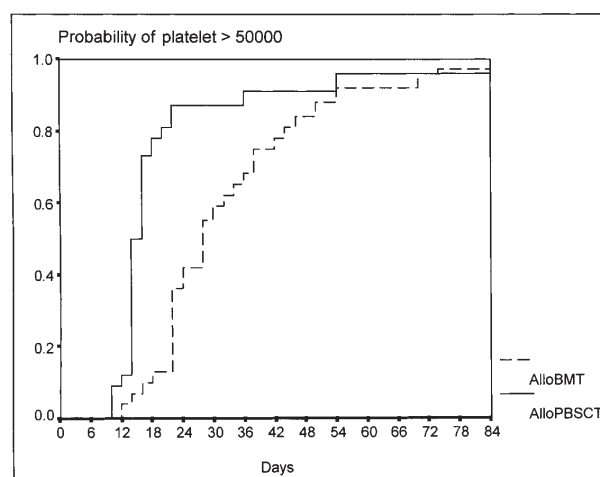


Figure 2. Platelet engraftment

In the alloBMT group, six patients relapsed after alloBMT (Table 2): three with AML, one with acute lymphoblastic leukemia (ALL), and two with CML. A profile of relapses in the alloBMT group is as follows: cytogenetic relapse in two patients with CML 23 and 34 months after transplantation. Of these, one is still alive 5 months after donor leukocyte infusion (DLI); the other died of persistent disease. Hematologic relapse was detected in three patients with AML 4, 16, and 20 months after transplantation (Table 5). One of these has achieved his second CR after receiving DLI and two died of persistent diseases. The patients with ALL died because of the progression of the disease.

In the alloBMT group, a total of 19 patients died of GVHD ($n=8$), graft failure ($n=1$), interstitial pneumonia ($n=2$), aplastic death ($n=4$), and relapse ($n=4$; Table 4). The causes of death in 15 patients in the alloPBSCT group were GVHD ($n=5$), relapse ($n=2$), aplastic death ($n=4$), graft failure ($n=1$), graft rejection ($n=1$), interstitial pneumonia ($n=1$), and hepatic failure ($n=1$, $p > 0.05$). Transplant-related

mortality at Day 100 was similar for both groups at 20% (8 of 40) vs. 17.5% (7 of 40, $p > 0.05$). In the alloBMT group, median DFS and OS were 34 (range 0–62 months) and 38 months (range 0.5–62 months), respectively. In the alloPBSCT group, median DFS and OS were 12 (range 0–34 months) and 12.5 months (range 0.5–34 months), respectively (Figs. 4 and 5). The estimated DFS in month 24 was 51.3% for alloBMT and 54.6% for alloPBSCT ($p = 0.81$) and the estimated OS in month 24 was 56.1% for alloBMT and 64.6% for alloPBSCT ($p = 0.92$).

DISCUSSION

Since the first primary allogeneic transplant utilizing PBSCs collected after G-CSF was reported by Russell *et al.* in 1993 [13], various studies have demonstrated the feasibility of using allogeneic peripheral stem cells collected after the administration of G-CSF from normal donors [2–5,11–14].

Table 3. Methods used to demonstrate engraftment origin and relapse

	CML	AML	Other	Total
Engraftment origin				
Sex mismatch ($n=33$)				
Conventional cytogenetics	8	2	—	10
FISH	8	2	—	10
Total	16	4	—	20 (60.0%)
Ph (>6 months, $n=24$); conventional cytogenetics				
	19	—	—	19 (79.1%)
ABO mismatch ($n=36$); group typing				
	17	12	2	31 (86.1%)
HLA polymorphism ($n=15$); VNTR				
	4	7	1	12 (80.0%)
Relapse ($n=9$)				
Hematological evaluation	1	5	1	7
Conventional cytogenetics	3	ND	0	3
VNTR	ND	2	0	2

FISH, fluorescence in situ hybridization; HLA, human leukocyte antigen; VNTR, variable nontandem repeats.

Table 4. Survival characteristics

	Group I (BMT)	Group II (PBSCT)	p
Alive	20/40	25/40	—
Dead	19/40	15/40	—
Loss of follow-up	1/40	—	—
Transplant-related mortality (first 100 days)	20% (8/40)	17.5% (7/40)	>0.05
Causes of death			
GVHD (total)	8	5	>0.05
Acute	5	0	—
Chronic	3	5	—
Relapse	4	2	>0.05
Early death	4	4	>0.05
Graft failure	1	1	>0.05
Graft rejection	0	1	>0.05
Interstitial pneumonia	2	1	>0.05
Hepatic failure	0	1	>0.05

The doses of G-CSF used for the priming of healthy donors varied during the early days of this treatment modality. In the literature, there is still no consensus as to the dosage of G-CSF ($2.5\text{--}15 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). In the current study, our donors in the alloPBSCT group received G-CSF in these ranges and at a median of $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. Platelet and neutrophil engraftments were significantly faster in the alloPBSCT group than in the alloBMT group, which is consistent with the data from current literature [13,15]. The fact that the harvest yields of stem cells were similar ($p = 0.07$), but that the alloPBSCT group retained more MNCs ($p = 0.04$) than the alloBMT group might be a reason for the faster engraftment. It has been suggested that $\text{CD}34^+$ cell count must be at least $2.5 \times 10^6/\text{kg}$ for successful engraftment [15]. Among all patients in the alloPBSCT group, one patient who received $<2 \times 10^6/\text{kg}$ $\text{CD}34^+$ cells died on the 12th day from sepsis with aplasia. Rapid engraftment in alloPBSCT patients resulted in fewer days on antibiotics and fewer days of fever. There were, otherwise, no statistical differences.

Consistent with our data, Pavletic *et al.* showed that the number of patients who spent days on antibiotics was not statistically greater in the alloBMT group than in the alloPBSCT group [16]. Pavletic *et al.* also demonstrated that the median transfusion of platelets and erythrocytes was higher in patients who were given transplants of alloBMT, but only platelet need was statistically less [16]. Bensinger *et al.* reported that the need for transfusions of RBCs and platelets was significantly higher in patients undergoing alloBMT in comparison to patients undergoing alloPBSCT [14]. In our study, the median units of platelet and erythrocyte transfusions were higher in the alloBMT group, but these differences were not statistically significant. There were no differences between the two groups regarding graft failure and transplant-related deaths.

In this study, the incidence of aGVHD was similar in both groups, but the incidence of cGVHD was higher in patients undergoing alloPBSCT than in those undergoing alloBMT, as in the preliminary study results from previous

Table 5. Organ involvement in cGVHD

	BMT (n=6)	PBSCT (n=25)
Liver	5 (83.3%)	21 (84.0 %)
Skin	5 (83.3%)	12 (48.0 %)
Eyes	1 (16.6%)	3 (12.0%)
Gastrointestinal tract	3 (50.0%)	6 (24.0%)
Hematopoietic	2 (33.3%)	7 (28.0%)
Pulmonary	1 (16.6%)	7 (24.0%)

work [17,18]. Most cases of cGVHD were *de novo* and appeared after CsA was tapered off, starting from the 6th month. Potential predictors of cGVHD, including donor age and pretransplant donor and recipient CMV serostatus, were similar in both groups, whereas patient age was higher in the alloPBSCT group (a factor associated with an increased risk of developing cGVHD) [19–21]. In addition, the higher incidence of cGVHD in patients receiving an alloPBSCT may also be related to the content of the infused MNCs and lymphocytes. As previously reported, the existence of prior aGVHD is another risk factor. In both groups, cGVHD developed more often in patients who had previously had aGVHD [21].

Initial studies reported an acceptable rate of cGVHD in patients receiving alloPBSCT compared with a historical group of alloBMT patients. Some groups with a longer follow-up period reported an increased incidence of cGVHD in patients undergoing alloPBSCT [22,23]. Storek *et al.* reported an increased incidence of cGVHD as the duration of follow-up time increased in patients with hematological malignancies undergoing alloPBSCT [22]. In that study, cGVHD developed at a median of 4 months (range) after transplantation. Storek *et al.* speculated that increased incidence of cGVHD in alloPBSCT patients may be related to an unmodified blood stem cell graft, which contains 2 logs more of T lymphocytes than an unmodified marrow graft [22]. In addition, Talmadge *et al.* reported that alloPBSCT patients had a significantly faster recovery of NK cells, $\text{CD}4^+$ T cells,

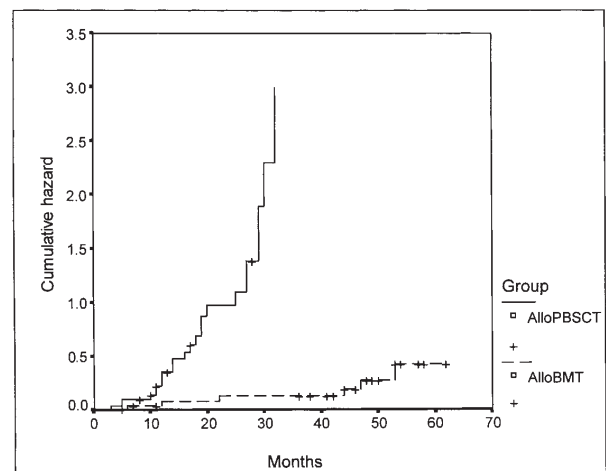


Figure 3. Cumulative hazard rate of cGVHD

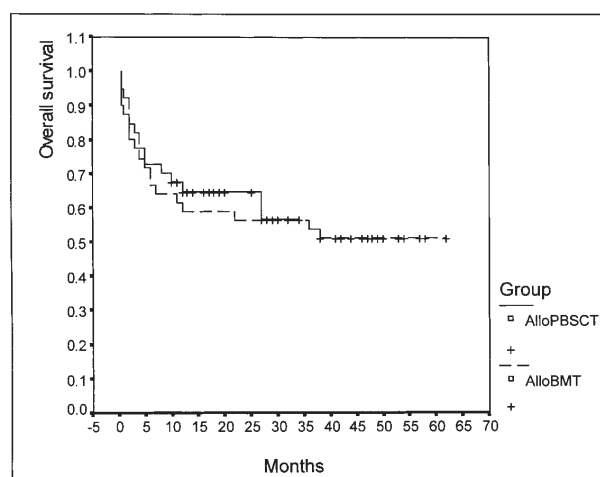


Figure 4. Overall survival analysis of patients

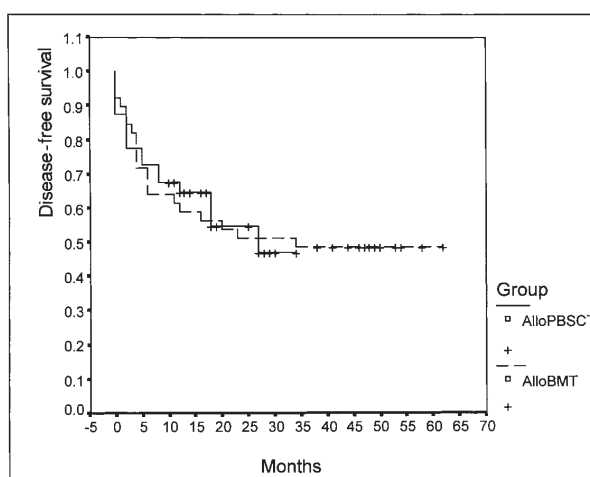


Figure 5. Disease-free survival at 24 months

and T-cell receptor $\gamma\delta$ [24]. As in the Talmadge *et al.* study, we found a gradual decrease of the CD4/CD8 cell ratio in the alloPBSCT group and an increase in the CD3⁺ cell counts. Reactions to minor histocompatibility antigens, which play an important role in the development of cGVHD, is mediated largely, though not exclusively, by CD8⁺ cells [25,26]. After transplantation, the decrease of the CD4/CD8 cell ratio and the increase in CD3⁺ cell counts may be responsible for cGVHD.

It has been demonstrated that GVHD is associated with an antileukemic effect, hence the lower incidence of relapse in patients who developed GVHD than in those who did not [27–29]. Some *in vivo* human studies showed less relapse in alloPBSCT [30]. In our study, the relapse rate was not statistically different between the two groups. Of the nine patients in the study who relapsed, one in both groups developed cGVHD before relapse. In addition, in the alloPBSCT group, one patient who relapsed had conditions that might explain this event: $<3 \times 10^6/\text{kg}$ of CD34⁺ cells, a longer time to transplantation, and, most significantly, transplantation at her second CR. DFS was significantly higher in alloBMT due to the prominent differences between follow-up periods in both groups.

In contrast to some current studies in which the liver is usually not the first organ involved in cGVHD, in the alloPBSCT group, the liver was the most affected organ from cGVHD in our study [22,23]. Despite the fact that the patients with liver cGVHD received megadoses of prednisolone, they did not respond to treatment and three patients died. Of these, two died due to cGVHD secondary to immunosuppression and pulmonary infection and failure; the third death was due to hepatic failure.

Using several methods, it is possible to evaluate chimerism following transplantation. For this purpose, ABO blood group antigen typing, sex chromosome analysis with FISH by using sex probes, and conventional cytogenetics have been used. Recently, polymerase chain reaction (PCR)-based VNTR, the most sensitive, rapid, and powerful technique, has been applied in this field [12]. We were able to use this technique for almost all alloPBSCT patients, and all

but three demonstrated CC. Most of the patients in both groups had donor type karyotype and blood group, which shows the success of transplantation. On the other hand, MC may represent minimal residual disease and predict hematological relapse as has been previously reported. At the time hematological relapse occurred, sequential VNTR analysis showed a conversion from CC to MC in two patients with AML. Another patient's VNTR analysis demonstrated rejection at the time she was in hematological CR and had the donor's blood group. She relapsed 5 months after the VNTR analysis. Of two patients with CML whose early phase VNTR analysis showed rejection, one achieved hematologic and cytogenetic remission and the donor's blood group, but the other never did. These findings support the idea that the presence of residual malignant cells of the recipient can only be detected by VNTR, which is a very sensitive molecular method. However, the results of VNTR do not clearly demonstrate forthcoming relapse in patients with CML in the early postengraftment phase.

In our study, we report the follow-up findings of alloPBSCT patients and their leukemic behavior based on various methods that have been extensively analyzed and published.

ACKNOWLEDGMENTS

The authors thank Alev Türker and Asuman Sunguroğlu, for their efforts in cytogenetic studies. We also thank Penelope Cumler for manuscript revision and Atilla Halil Elhan for statistical analysis.

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